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DEVELOPMENT AND VALIDATION OF COST EFFECTIVE RP-HPLC ASSAY METHOD FOR DETERMINATION OF METAXALONE

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ABSTRACT

Most of the analytical method required, fast and reliable analytical technique as per current requirement of pharmaceutical industry. Hence a simple rugged and cost effective method was developed and validated for Metaxalone. By using this HPLC method, the time for analysis is reduced sensationally as compared to other method published in most of the literatures published. The method was set on Ascentis express C8 column (10cm x 4.0mm, 2.7µ) using 0.1% Triethylamine as a buffer (pH 3.0 with per chloric acid) and acetonitrile as organic modifier. The flow rate was set at 1.0ml\min and analysis time was 4 minutes with gradient elution. The detection was conducted at 210nm. The method was validated as per International Conference of Harmonization (ICH) Guidelines in terms of Specificity linearity and range, precision and robustness. Sample and standard concentration of metaxalone was 0.5mg/ml. linearity of standard was covered from 0.4mg/ml to 0.6mg/ml.

Keywords – *Metaxalone, HPLC, Reversed phase, Validation*

1. INTRODUCTION

Metaxalone is used for the treatment of muscle relaxant and relieve pain caused by strain, sprain and other musculoskeletal conditions. The metabolism of metaxalone involves the liver cytochrome P450 system. Based on the information in the labelling, patients receiving metaxalone therapy and physicians prescribing metaxalone are directed to take precaution when administering it with other medications involving the P450 system.

Because of potential for side effects, this drug is considered high risk in the elderly. As of 2015 the cost for a typical month of medication in the United States is 100 to 200 USD [1-8].

Several HPLC methods have been reported for determination of metaxalone either in bulk drug or in combination of another drug. [5-8] Previous published method having runtime of around 15 minutes however there is no method available with runtime of 4 minutes. Therefore, aim of this study was to develop and validate the assay method for determination of metaxalone (Figure 1).

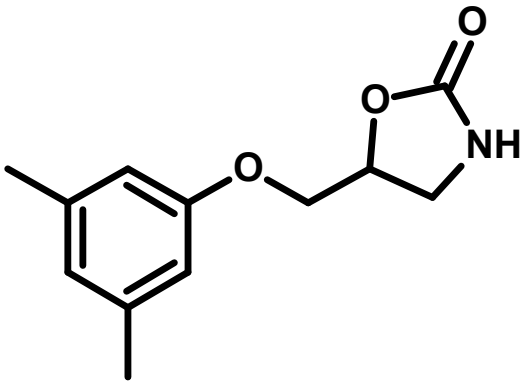

Fig. 1: Chemical structure of Metaxalone

2. MATERIALS AND METHODS

2.1 Materials and reagents

Gift samples of Metaxalone were received from R & D centre Ajanta laboratories, Mumbai, India. HPLC grade Methanol, Acetonitrile, trimethylamine and perchloric acid were purchased from Rankem, fisher scientific and Merck. Millipore Milli Q grade HPLC water used for mobile phase preparation.

2.2 Instrument/Equipment

The Agilent HPLC 1100 series liquid chromatography with Binary pump with auto sampler, PDA was used for method development and validation. The output signal was monitored and processed through chromeleon software.

2.3 Chromatographic conditions

The chromatographic separation was achieved on Ascentis express C8 column, 100mm x 4.0mm, 2.7µ column. 0.1%trimethylamine buffer was prepared and pH adjusted to 3.0 with perchoric acid. Mixture of Mobile Phase Buffer and Acetonitrile was used in ratio of 40:60 v/v. Methanol were used for sample preparation and standard preparation. The flow rate of mobile phase was kept at 1.0ml/min with an isocratic program. The column temperature was maintained 40°C and the chromatogram was monitored at wavelength of 210nm. The injection volume was 5µ (Figure 2-4)

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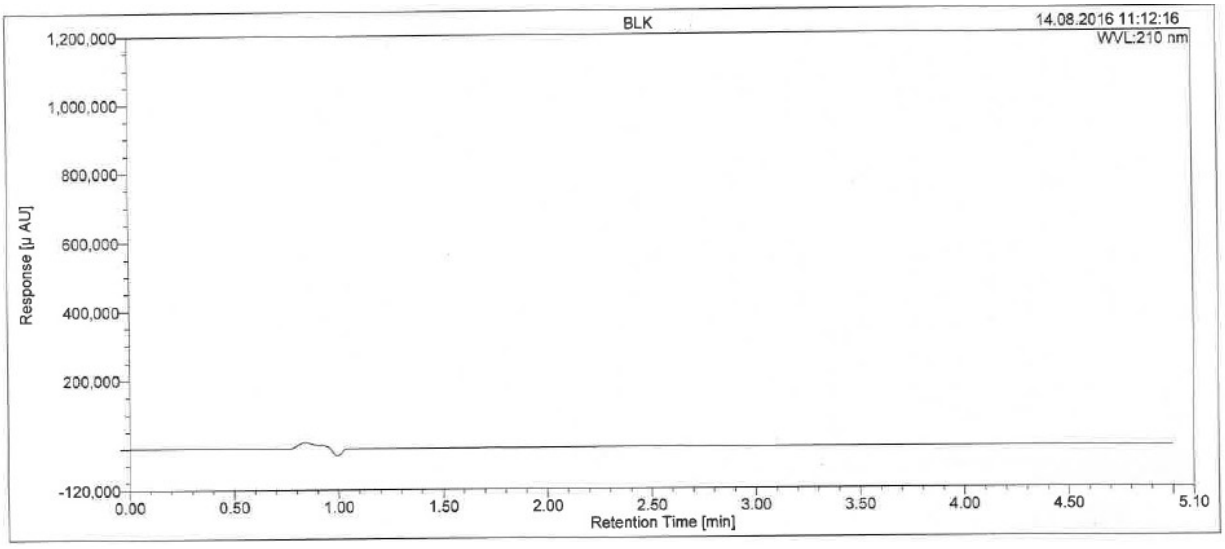

Fig. 2: Blank chromatogram

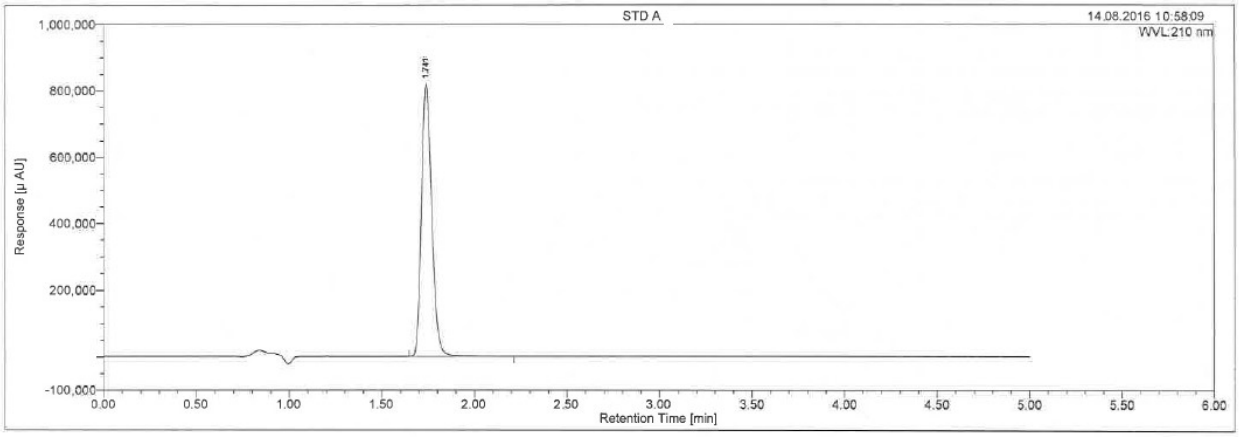

Fig.3: Standard chromatogram of Metaxalone

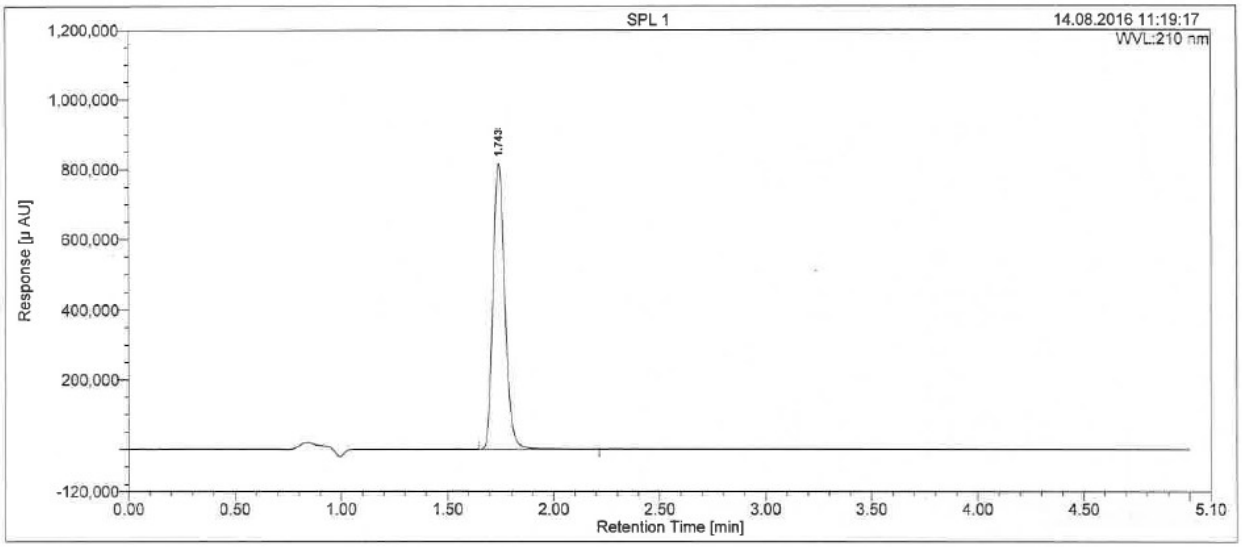

Fig. 4: Sample chromatogram of Metaxalone

2.4 Preparation of standard and sample solutions of Metaxalone

The standard and sample stock solutions were prepared with concentration of 0.5mg/ml by dissolving an appropriate amount of metaxalone in methanol diluent.

2.5 Method Validation

The Method validation were carried out as per ICH Guidelines with parameter of Specificity linearity and range, precision and robustness. The system precision was determined by measuring inter-day and intra-day variation. The % RSD was calculated for six replicate injections and found less than 2 %.(Table 1 and table 2)

2.6 Linearity

The Linearity metaxalone were determined by plotting three different concentration of calibration curves. First and last level of linearity were injected three replicate and limit level were injected in duplicate and %RSD calculated. The calibration curve for metaxalone was plotted for peak areas versus concentrations of analyte. Regression coefficient was calculated at 3 levels, Viz., 80%, 100% and 120%. (Figure 5)

2.7 Robustness and solution stability

Robustness of the method were checked by varying the pH of mobile phase (± 0.05) and temperature (± 0.05ºC). Solution stability and mobile phase stability were studied by intermediate precision study.

Table 1: System precision (Standard)

Replicate STD A (500 ppm)	Retention time	Area
1	1.735	3146883.00
2	1.737	3307341.00
3	1.741	3180336.00
4	1.743	3180503.00
5	1.741	3177869.00
6	1.741	3179491.00
Mean	1.74	3195403.83
SD	0.00	56380.78
% R.S.D.	0.2	1.8

Table 2: Sample precision

Spl.No.	Spl Wt. (mg)	Spl Area	Std A Wt. (mg)	Std A Mean Area	Assay
Sample 1	50.13	3178822.0	50.13	3195403.83	99.48
Sample 2	50.15	3173082.0			99.26
Sample 3	50.09	3173528.0			99.39
Sample 4	50.17	3173500.0			99.24
Sample 5	50.08	3171892.0			99.36
Sample 6	50.11	3169087.0			99.22
				Mean	99.38
				SD	0.11
				%RSD	0.11

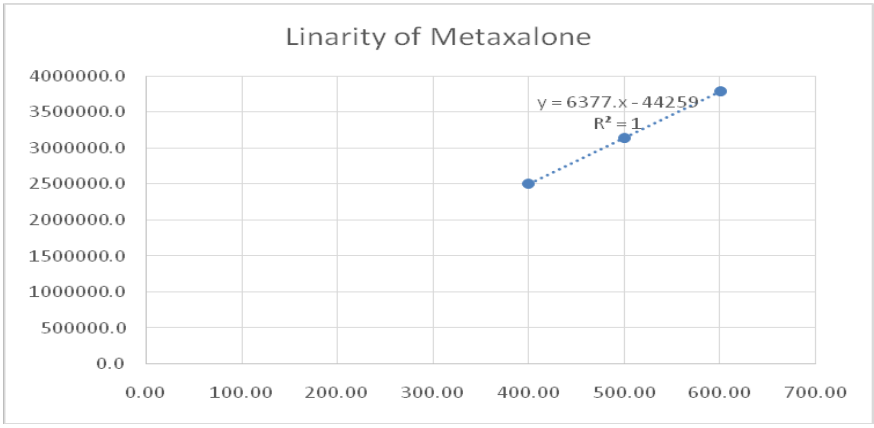

Fig. 5: Linearity of metaxalone

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic condition

The method was optimized to develop cost effective, accurate and less time consuming HPLC method for determination of Metaxalone for routine analysis. The wave-length selected was 210 nm, since the metaxalone was having good response at 210nm. During assay method development of metaxalone different HPLC column were tried with different mobile phase. The HPLC column such as Zorbax C8 column 250mm x 4.6mm, 5µ, peak was eluted at retention time of 11 minutes using buffer 0.1% Triethyl amine pH adjusted to 3.0 with perchloric acid. Ratio of Mobile phase was 50:50 v/v. Different column having different column chemistry with different dimension were tried (Inertsil C18 250mm x 4.6mm, 5µ) but not received the achievable results. Using Ascentis express C8 column with dimension of 100mm x 4.0mm, 2.7µ, good peak shape observed and Retention time was 1.6. Buffer was 0.1% trimethylamine pH adjusted to 3 with perchloric acid. Mixture of Mobile phase containing Buffer: Acetonitrile in ratio of 40:60 v/v. flow rate was 1.0ml/minutes and wavelength 210nm. Column oven temperature kept at 40ºC for asymmetric peak. The %RSD was calculated and observed at less than 2.0%

3.2 Method Validation

3.2.1 Specificity

Specificity of the method is its ability to detect and separate the impurities present in the drug. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug and impurities present in drug. Peak passed the peak purity test.

3.2.2 Linearity

Linearity of the method was checked by preparing solutions at three concentration levels of 0.4mg/ml (Level 1), 0.5mg/ml (Level 2) and 0.6mg/ml (Level 3. The mean area responses recorded for each level were plotted against concentration. The correlation coefficient for metaxalone as found to be 0.999, which indicates good linearity.

3.2.3 System and method precision

The system for assay method was checked for repeatability. The sample and standard was prepared and injected six times. The % RSD was found to be less than 2.0% for system precision.

4. CONCLUSION

The selective, stability indicating, cost effective and simple reverse phase HPLC method was developed and validated for metaxalone. This method can be used for routine quality control analysis.

5. ACKNOWLEDGEMENTS

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